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Article

Pre-clinical interaction of ayahuasca, a brew used in spiritual movements, with morphine and propofol

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Ayahuasca is a beverage with psychoactive properties used in religious and ceremonial rituals by some religious groups. The main active components of ayahuasca are dimethyltryptamine and the harmala alkaloids with β-carboline structure acting as monoamine oxidase A inhibitors. This combination produces a pronounced activation of serotonergic pathways and presents potential interaction with other psychotropics. The objective of this study was to investigate the possible interactions between ayahuasca and agents employed in general anesthesia. The pharmacological interactions between ayahuasca and morphine or propofol were evaluated in mice using doses of 12, 120 and 1200 mg/kg (0.1 to 10 times the average dose consumed by humans in religious rituals). Ayahuasca alone showed an antinociceptive effect in the writhing and formalin tests, and intensified the analgesic effect of morphine in the hot plate test. Concerning the pharmacological interactions between ayahuasca and propofol, the results were opposite; ayahuasca intensified the depressant effect of propofol in the rotarod test, but decreased the sleeping time induced by propofol. These set of results showed the occurrence of some interactions between ayahuasca and the drugs morphine and propofol, possibly by both pharmacokinetics and pharmacodynamics mechanisms.

Keywords: Ayahuasca. Analgesic effect. Pharmacological interactions. Morphine. Propofol. β-Carbolines.

INTRODUCTION

Ayahuasca is a beverage with psychoactive properties prepared by decoction of two Amazonian plants, the vine of *Banisteriopsis caapi* (Spruce ex Griseb.) CV Morton (Malpighiaceae) and the leaves of *Psychotria viridis* (Ruiz & Pav.) (Rubiaceae) utilized in ceremonial contexts of groups like Santo Daime, União do Vegetal, among others. The religious use of ayahuasca is legally accepted in Brazil and also occurs in the United States and Europe (Labate, Feeney, 2012). Participants generally meet two to three times per month for the realization of a religious cult and they consume one or two cups of ayahuasca brew at the beginning of the ritual, but the amount of ayahuasca taken in a session may vary among the individuals and depending on the concentration of the beverage (McKenna, 2004). There is no official statistics about ayahuasca misuse, but it is reported that its use has expanded on the last decades and it is present in at least 38 countries (Labate, Feeney, 2012; Domínguez-Clavé *et al*., 2016).

The main active components of ayahuasca are *N,N*-dimethyltryptamine (DMT) and the harmala alkaloids with β-carboline structure, harmine, harmaline, tetrahydroharmine (THH), and harmol, as well harmalol in trace amounts (Callaway, Brito, Neves, 2005). DMT is present in the leaves of *P. viridis* and, when taken orally, it is rapidly inactivated by monoamine oxidase A (MAO-A) in the bowel and liver, which is avoided with the combination of β-carbolines present in *B. caapi* acting as MAO inhibitors (MAOI) (McKenna, Towers, Abbott, 1984). DMT is a potent 5-HT_{1A}, 5-HT_{2A} and 5-HT_{2C} receptor agonist and also interact with other molecular targets (Frecska, Bokor, Winkelman, 2016; Domínguez-Clavé *et al*., 2016). In

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addition, THH is believed to act as selective inhibitor of serotonin reuptake (Frecska, Bokor, Winkelman, 2016). The result of this interaction is a pronounced stimulation of serotonergic pathways leading to possible therapeutic potential, but also to potential interactions with other central nervous system drugs (Domínguez-Clavé *et al*., 2016).

The anxiolytic properties of ayahuasca are described both in preclinical and clinical studies (Santos *et al*., 2007; Santos *et al*., 2016) and may be related with its serotonergic activity. According to information from members of the ayahuasca groups, it is a common procedure to drink a glass of ayahuasca before surgery to reduce anxiety and promote calmness (R. Guiso, personal communication), which makes relevant to evaluate possible interactions between the beverage and agents used in anesthesia. It is noteworthy that there is no medical literature assessing the safety of anesthetic procedures in patients who use ayahuasca.

It was shown that ayahuasca components interfere with cerebral levels of monoaminergic and aminoacidergic neurotransmitters, including GABA (Castro-Neto *et al*., 2013). Since some selective inhibitors of serotonin reuptake, antipsychotics and other psychotropics are metabolized by CYP2D6 that also metabolizes harmine and harmaline, the concomitant use of these substances may be problematic and should be avoided (Callaway, Grob, 1998; Callaway, 2005; Jiang *et al*., 2013). Moreover, it is known that MAOI may increase the depressant and analgesic effects of morphine (Kitanaka, Kitanaka, Takemura, 2006; Stockley, 2007), but there is no information of possible interactions with MAOI and propofol (Micromedex, 2016). In this study, mice were pretreated with ayahuasca and then treated with morphine or propofol to verify a possible pharmacological interaction among these agents.

MATERIAL AND METHODS

Animals

Male Swiss mice, 3-4 months of age, weighing 30-50 g, were obtained from the Department of Psychobiology, UNIFESP. The animals were kept in an isolated room with a light/dark cycle of 12 h (7:00 a.m.–7:00 p.m.) at constant temperature (23 ± 2°C) and fed *ad libitum*, except during testing. At the conclusion of the experiments, the animals were euthanized in a CO_2 chamber. For the experiment of intestinal transit, the mice were killed by cervical dislocation. This study was approved by the Ethics Committee of UNIFESP (protocol 1494/06) following the standards recommended by the "International Guiding Principles for Biomedical Research Involving Animals" Geneva, 1985.

Drugs, reagents and the preparation of ayahuasca liophilizate

Commercial samples of propofol (Propovan®) and morphine sulfate (Dimorf®) were donated by Cristália Laboratory (São Paulo, Brazil). Acetic acid, charcoal, gum arabic and formaldehyde were purchased from Labsynth (São Paulo, Brazil). Propofol and morphine were diluted in saline and administered by intraperitoneal route.

The sample of ayahuasca (AYA) was produced in Manaus (Brazil) and donated by the Associação Benevolente Luz de Salomão according to the ritualistic protocol of this religion. The sample was concentrated and lyophilized (yield of 12%). A glass of 70 mL of ayahuasca (average volume ingested during rituals of this entity) was dried producing 8.4 g of solid. Therefore, a man of 70 kg (middle-weight) receives the equivalent of 120 mg/kg (8.4 g/70 kg). This dose was chosen as the median unitary dose (1X). Doses equivalent to 0.1X (12 mg/kg), the ritualistic 1X (120 mg/kg) and 10X (1200 mg/kg) were chosen for testing in order to increase the chances to observe the behavioral effects, since humans and rodents possess different metabolism, making the dosage transposition complicated.

Determination of the active constituents

The main active constituents found in ayahuasca (dimethyltryptamine, harmine, harmaline and tetrahydroharmine) were determined as previously described by Pires *et al*. (2009a).

In summary, the following procedure was performed: a sample solution containing the ayahuasca sample (0.5 mL), borate buffer (3.0 mL, pH 9.0) and the internal standard diphenhydramine (100 µL of a solution of 1.0 mg/mL) was loaded onto a solid-phase extraction C18 cartridge mounted on a vacuum manifold and conditioned with methanol (2.0 mL), deionized water (1.0 mL) and borate buffer (pH 9.0, 2.0 mL). The loaded cartridge was further washed with deionized water (1.0 mL) and a solution of acetonitrile-water (1:9, 1.0 mL). After drying the cartridges under full vacuum for 7 min, the elution of analytes was performed with methanol (2.0 mL). Then, 1 µL of this solution was injected in a Agilent gas-chromatograph, model 6890, equipped with a nitrogen–phosphorous detector and a 7683 series automatic injector (Little Falls, DE, USA). The analytes

were identified based on a comparison of its relative retention time with the corresponding values of standards assayed in the same chromatographic run. Quantification was based upon the ratio of the integrated peak area to the internal standard.

Initial pharmacological screening

Groups of five mice each were treated intraperitoneally (i.p.) or orally (p.o.) by gavage with doses of 12, 120 and 1200 mg/kg of ayahuasca. The controls received the vehicles saline (i.p.) or water (p.o.). The animals were observed at intervals of 30 min for 4 h following treatment according to the protocol routinely employed by our group (Pires *et al*., 2009b; Bezerra *et al*., 2010). We observed the presence or absence or several signs, such as tremor, palpebral ptosis, muscular tonus, locomotor activity, ataxia, stereotypy, miction, defecation, among others. This experiment was used to establish the doses for subsequent tests and to disclose an initial profile of ayahuasca activity.

Tests to evaluate interaction between ayahuasca and propofol

A possible interaction between ayahuasca and propofol was evaluated in two behavioral tests: rotarod – to assess the motor coordination; and sleeping time – sedative / hypnotic effect. The doses of propofol employed in each test were chosen based on literature data and pilot studies (data not shown).

Rotarod test

The performance of mice was measured by evaluating the time they remained on the rotating bar of the rotarod apparatus (AVS Project, Brazil) at a constant speed of 12 rpm. The animals were previously selected 24 h before by eliminating those that could not stay on the bar for 60 s (Bezerra *et al*., 2010). First, we evaluated the effect of ayahuasca (120 and 1200 mg/kg, p.o.) or propofol (25, 50 and 100 mg/kg, i.p.) isolated on the motor coordination of mice (n=5/group; pilot study). Then, groups of ten mice treated p.o. with water (control) or ayahuasca 1200 mg/kg, 30 min before propofol (100 mg/kg) or saline (both i.p.) were evaluated on the rotarod apparatus at the times 0 (basal), 30, 60 and 120 min. The dose of 1200 mg/kg of ayahuasca was chosen in order to increase the chance of observing propofol-ayahuasca interaction, since neither 120 nor 1200 mg/kg affected the mice motor coordination per se.

Sleeping time test

The doses of propofol were chosen based on pilot study and those described in the literature (Anwar, Abdel-Rahman, 1998; Xu, Duan, Zeng, 2004). At the pilot study, the sleeping time was evaluated for mice treated i.p. with several doses of propofol (n=5/group). Then, groups of 7-10 mice each were pretreated (p.o.) with water (control) or ayahuasca (120 and 1200 mg/kg) followed by treatment with propofol (140 or 175 mg/kg, i.p.) 30 min later. The latency time (time elapsed between propofol administration and the loss of the righting reflex) and the sleeping time (time required for the animal to recover the righting reflex) were recorded (Bezerra *et al*., 2010).

Tests to evaluate interaction between ayahuasca and morphine

Three tests were employed to assess both a possible antinociceptive action of ayahuasca and its possible interaction with morphine. A fourth test evaluated the effects of morphine, ayahuasca and their interaction on intestinal transit.

Hot plate test

Groups of 7-10 mice each were treated p.o. with water (control) or ayahuasca (120 and 1200 mg/kg) and after thirty minutes treated by i.p. route with morphine (10 mg/kg) or saline and then evaluated at 0 (basal), 30, 60, 120 and 240 min according to Pires *et al*. (2009b). Each mouse was placed individually on a hot plate (Ugo Basile, Italy) maintained at 55 ± 1 °C, and the elapsed time required for the animal to produce the characteristic responses to painful stimuli, such as jump or licking their paws, was recorded. Thirty seconds was considered the cut-off time to avoid tissue damage.

Acetic acid writhing test

Groups of 10 mice each were treated p.o. with water (control) or ayahuasca (120 and 1200 mg/kg). Thirty minutes later, morphine (1 mg/kg) or saline were administered i.p. After an additional 30 min, 0.8% acetic acid was injected (i.p.), and the number of writhings (contractions of the abdomen with distension of the hind legs) was counted over a period of 20 min (Pires *et al*., 2009b).

Formalin test

In the first experiment, mice (n= 5-7 per group) were

treated p.o. with water (control), ayahuasca (12, 120 and 1200 mg/kg) or morphine (10 mg/kg, i.p.). In the second experiment, mice (n= 7-9 per group) received ayahuasca 120 mg/kg or water (p.o.) and 30 min later morphine (1 mg/kg) or saline by i.p. route. In both experiments, the test consisted of the subplantar injection of 20 µL of formalin (2.5%) in the right rear paw 30 min after the last treatment. Behavior was then measured in two periods. The animals were observed to evaluate the reaction to pain, measured as the time spent licking the paw, during the first phase (neurogenic; 0-5 min) and the second phase (inflammatory; 15-35 min) (Pires *et al*., 2009b).

Intestinal transit test

Groups of five mice each, fasted for 24 h, were treated p.o. with water (control) or ayahuasca (120 and 1200 mg/kg), and after 30 min with morphine (5 mg/kg) or saline by i.p. route. Thirty minutes later, mice were given 0.35 ml (p.o.) of an aqueous suspension of 10% activated charcoal in 5% gum arabic. Fifteen minutes later, each animal was killed by cervical dislocation, the small intestine removed and the distance that the charcoal had traveled from the pylorus was measured and expressed as the percentage of the total length of the small intestines (Pires *et al*., 2009b).

Statistical analysis

The results are expressed as the mean ± standard error of mean (SEM). One-way ANOVA followed by Duncan's test was employed to evaluate data with normal distribution: writhes, formalin, intestinal transit and sleeping time tests. Hot plate test (repeated measure) was analyzed by two-way ANOVA followed by Newman Keuls test. Motor coordination was analyzed by the Kruskal-Wallis followed by Mann-Whitney test after found a non-normal distribution. A level of significance of 5% ($p<0.05$) was adopted.

RESULTS

Determination of active constituents

Tetrahydroharmine (1.67 mg/mL) was the major constituent found in the ayahuasca sample followed by dimethyltryptamine (0.38 mg/mL), harmine (0.32 mg/mL) and harmaline (0.12 mg/mL).

Initial pharmacological screening with ayahuasca

Intraperitoneal doses of 120 mg/kg and 1200 mg/kg of ayahuasca, under qualitative observation, produced decrease in locomotion and grooming, and increase of sensibility of mice to sound. Intense tremor was observed in the mice treated with 1200 mg/kg. These alterations did not occur with the oral route or were induced in minor intensity. No death was observed.

Rotarod test

The pilot study with ayahuasca and propofol isolated showed that ayahuasca at 120 and 1200 mg/kg or propofol at 25 and 50 mg/kg did not interfere with mice motor coordination, but propofol at dose of 100 mg/kg reduced the time on rota rod (data not shown). Further, we evaluated the effect of ayahuasca 1200 mg/kg and Propofol 100 mg/kg isolated and in association.

As can be seen in Figure 1, ayahuasca 1200 mg/kg, by oral route, did not interfere with mice's capacity to maintain themselves on the rotating bar. On the other hand, propofol (100 mg/kg, i.p.) greatly reduced the performance of the animals as all of them fell from the rod in less than 20 s at 15 min after drug administration, but this effect was short-lived as the mice recovered the performance 15 min later (at 30 min measure). Furthermore, the group treated with ayahuasca + propofol presented motor incoordination at 15 and 30 min, when the effect was no more present on propofol group.

Sleeping time

The pilot study showed that propofol at doses between 100 and 200 mg/kg was able to induce sleep in mice (data not shown). As seen in Figure 2 the latency time to sleep was similar among the groups administered with propofol (140 and 175 mg/kg, i.p.). Ayahuasca (120 and 1200 mg/kg, p.o.), somewhat decreased propofol sleeping time, although significance was reached only with the dosage of 120 mg/kg of ayahuasca plus 175 mg/kg of propofol. However, this effect was not confirmed with the larger dose of ayahuasca (1200 mg/kg).

Hot plate test

As can be seen in Figure 3, morphine induced the expected antinociceptive effect in mice at 30 min when compared with the basal latency, although this effect did not reach significance in the following measurements. Ayahuasca alone (120 and 1200 mg/kg, p.o.) did not change the response time of mice to the hot plate test. The association of ayahuasca (1200 mg/kg) plus morphine (10 mg/kg) was able to induce the antinociceptive effect at

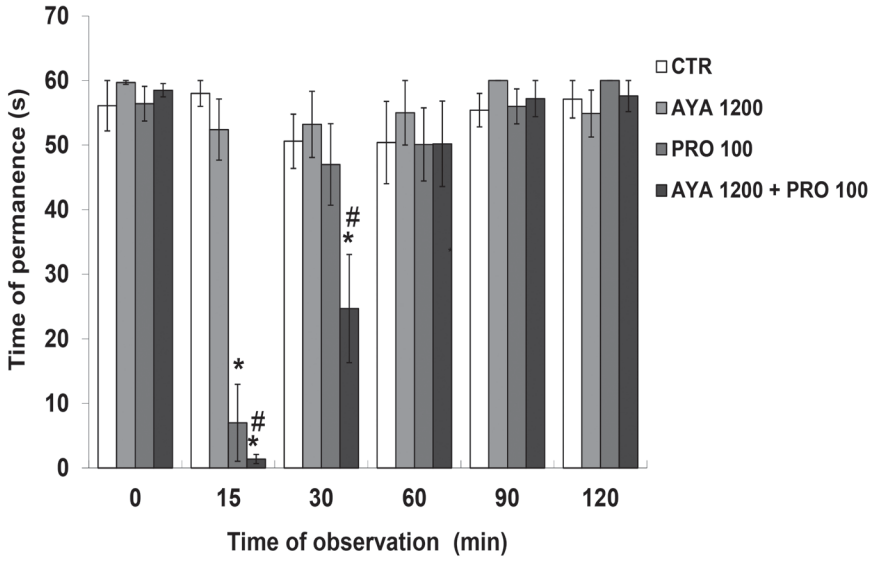

FIGURE 1 - Effect of ayahuasca (AYA, 1200 mg/kg, p.o.) isolated or in association with propofol (PRO, 100 mg/kg, i.p.), on the performance time of mice on the rotating bar. Results are expressed as the mean ± SEM (n=10). (*) $p<0.05$: statistically different from control; (#) $p<0.05$: statistically different from AYA 1200. Kruskal-Wallis followed by Mann-Whitney.

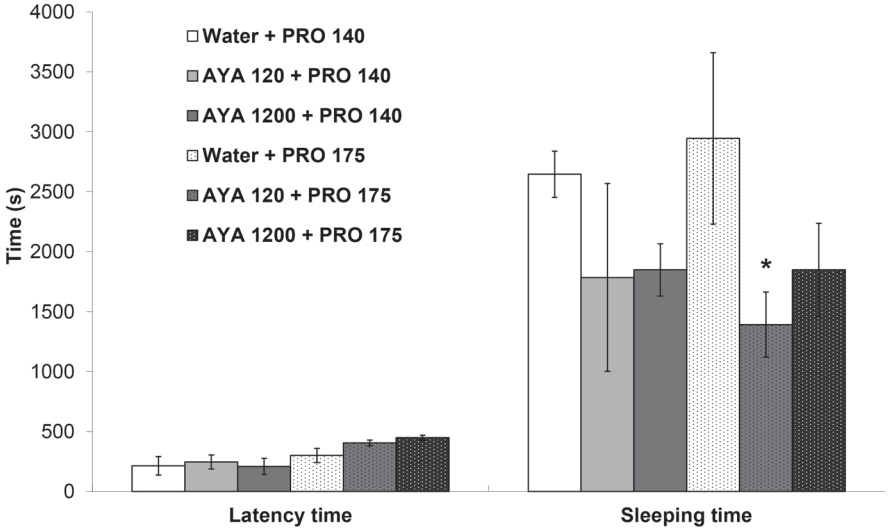

FIGURE 2 - Effect of ayahuasca (AYA, 120 and 1200 mg/kg, p.o.) on the latency time and sleeping time produced by propofol (PRO, 140 and 175 mg/kg, i.p.) in mice. Results are expressed as the mean ± SEM (n=7-10). (*) $p<0.05$: statistically different from group water + propofol 175 mg/kg. ANOVA followed by Duncan's test.

30, 60 and 120 min of observation, while the association with the dose of 120 mg/kg only reached significance at 120 min. Additionally, the association of ayahuasca (both doses) with morphine significantly increased the latency time at 120 min compared with morphine group or with ayahuasca alone (columns 5 and 6, Figure 3).

Acetic acid writhing test

Morphine (1 mg/kg, i.p.) significantly reduced the numbers of writhes induced by acetic acid when compared to control mice (Figure 4). Ayahuasca (120 mg/kg, p.o.) produced no effect per se and did not interfere with the morphine analgesia. However, ayahuasca (1200 mg/kg, p.o.) produced a significant reduction of writhes (65%) when compared to control, and the association of ayahuasca 1200 mg/kg plus morphine 1 mg/kg showed the most potent analgesic effect (column 6 of Figure 4).

Formalin test

Figure 5a shows that 10 mg/kg of morphine

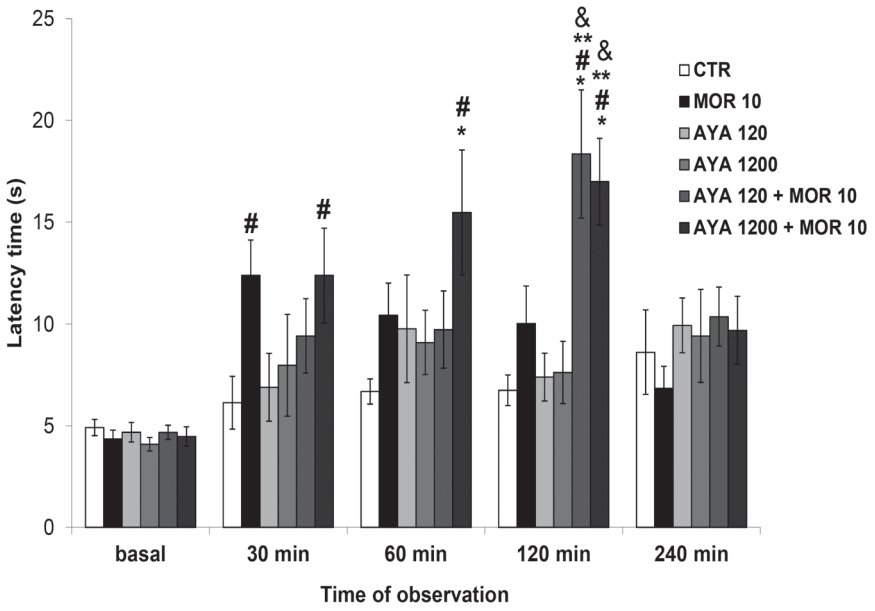

FIGURE 3 - Effect of ayahuasca (AYA, 120 and 1200 mg/kg, p.o.) associated with morphine (MOR, 10 mg/kg, i.p.) on the latency time of mice responding to the thermal stimulus. Results are expressed as the mean ± SEM (n=7-10). (*) $p<0.05$: statistically different from control; (#) $p<0.05$: statistically different from corresponding basal; (**) $p<0.05$: statistically different from morphine; (&) $p<0.05$: statistically different from corresponding dose of ayahuasca at the same observation time. 2-way ANOVA followed by Newman Keuls test.

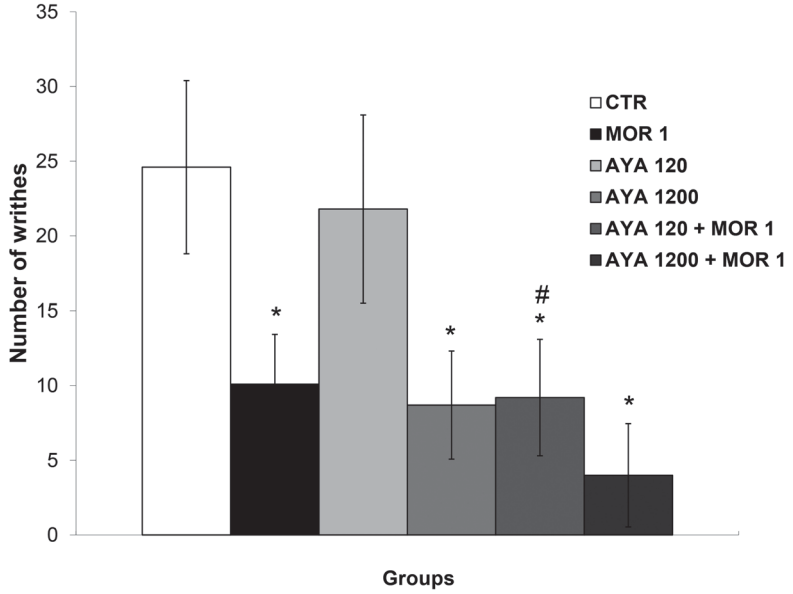

FIGURE 4 - Effect of ayahuasca (AYA, 120 and 1200 mg/kg, p.o.) and its association with morphine (MOR, 1 mg/kg, i.p.) on the number of writhes induced by acetic acid (0.8%, i.p.). Results are expressed as the mean ± SEM (n=10). (*) $p<0.05$: statistically different from control; (#) $p<0.05$: statistically different from AYA 120. ANOVA followed by Duncan's test.

(i.p.) significantly decreased paw licking during the 1st and 2nd phases by, respectively, 74 and 99%. The groups of mice treated p.o. with 12, 120 or 1200 mg/kg of ayahuasca also showed significant dose-response decrease in the number of writhes in the first phase by, respectively, 35%, 42% and 75% in relation to the control group. On the other hand, measures taken at the second phase revealed that only the largest dose of ayahuasca (1200 mg/kg) produced a significant effect (reduction of 95%).

Figure 5b shows the results of the combination of the sub-effective dose of morphine and ayahuasca. Morphine (1 mg/kg, i.p.) alone did not diminish significantly the licking time on the first phase, while ayahuasca alone (120 mg/kg, p.o.) or in combination with morphine (1 mg/kg) caused a reduction in paw licking during the first phase by 30% and 38%, respectively. There was no difference among the groups in the second phase, although the mice of morphine group showed a tendency of reduction (34%) on paw licking response.

Intestinal transit test

Morphine (5 mg/kg, i.p.), as expected, reduced intestinal motility by about 60%, as shown in Table I. Both dosages of ayahuasca were not able to significantly alter intestinal transit or either to interfere with morphine effect.

DISCUSSION

Several interactions between drugs and herbal

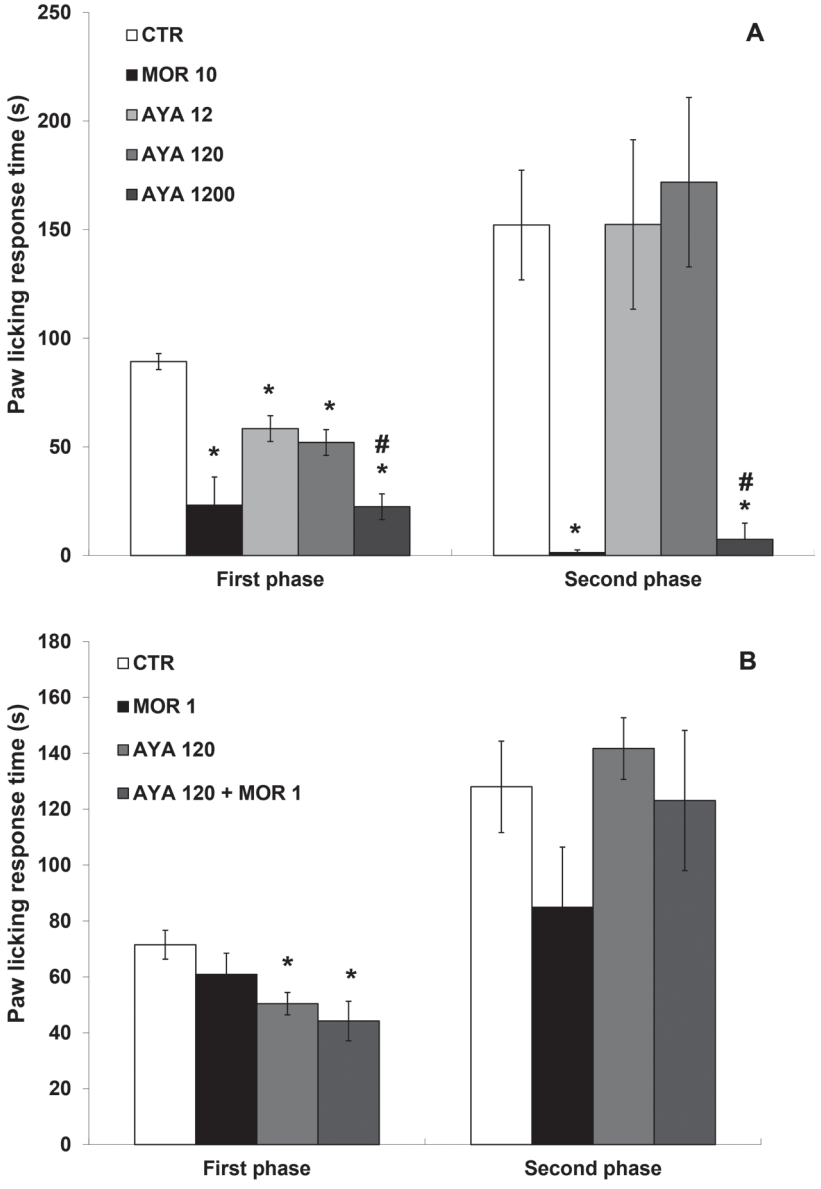

FIGURE 5 - Effect of ayahuasca and morphine on the time that mice spent licking the paw after formalin 2.5% subplantar injection. A) Ayahuasca (AYA, 12, 120 and 1200 mg/kg, p.o.) and morphine (MOR, 10 mg/kg, i.p.) B) Ayahuasca (AYA, 120 mg/kg, p.o.), morphine (MOR, 1 mg/kg, i.p.) and the respective groups of association. Results are expressed as the mean ± SEM (A: n=5-7; B: n=9-10). (*) $p<0.05$: statistically different from control; (#) $p<0.05$: statistically different from AYA 12 and AYA 120. ANOVA followed by Duncan's test.

TABLE I - Effect of ayahuasca (120 and 1200 mg/kg, p.o.) and its association with morphine (5 mg/kg, i.p.) in the distance traveled by activated charcoal in the small intestine (percentage of the total length). Results are expressed as the mean ± SEM (n=5)

Treatment and doses (mg/kg)	Intestinal transit (%)
Control (water + saline)	73.1 ± 8.4
Water + morphine 5	29.5 ± 2.9*
Ayahuasca 120 + saline	55.9 ± 8.6
Ayahuasca 1200 + saline	64.7 ± 1.9
Ayahuasca 120 + morphine 5	27.3 ± 5.2*
Ayahuasca 1200 + morphine 5	29.6 ± 4.2*

* $p<0.05$: statistically different from control. ANOVA followed by Duncan's test.

medicines have been reported, such as *Hypericum perforatum* and *Ginkgo biloba* (Fugh-Berman, 2000; Roby *et al*., 2000; Umegaki *et al*., 2007) and with foods, such as the juices of grapefruit or apples (Farkas, Greenblatt, 2008; Nowack, 2008), which interfere with the P_{450} enzyme system. The MAOI β-carbolines present in ayahuasca interfere with the absorption and metabolism of MAO substrates, but the effect of ayahuasca on drugs metabolized by other enzyme system (as P_{450}) is poorly known. It was hypothesized that harmaline can alter the metabolic route of 5-methoxy-N-N-dimethyltryptamine (5-MeO-DMT), an analogue of DMT, leading to CYP_{2D6} metabolism (alternative route) and increasing the production of bufotenine which seems to be more active than 5-MeO-DMT (Jiang *et al*., 2013; Halberstadt, 2016). However, 5-MeO-DMT is scarcely present in ayahuasca; it was found in trace amounts in some studies, but it was not detectable in most studies with different ayahuasca preparations (McKenna, 2004; McIlhenny et al, 2009; Pires, Oliveira, Yonamine, 2010). Nevertheless, the existence of an alternative route for DMT metabolism under MAO inhibition by β-carbolines was previously reported (Riba *et al*., 2012).

The contents of β-carbolines and DMT in different ayahuasca samples may vary considerably depending on the species and proportion of parts used, method of preparation, time cooking, among other aspects (Rivier, Lindgren, 1972; McKenna, 2004; Callaway, Brito, Neves, 2005; Callaway, 2005). It is known that several species morphologically similar are employed on the tea preparation with alkaloid profiles very distinct (Callaway, 2005; Pires, Oliveira, Yonamine, 2010). Most samples of ayahuasca contain predominant amounts of harmine and tetrahydroharmine, while harmaline, harmalol, harmol and other β-carbolines are found in low concentration (Callaway, 2003). The concentrations of DMT and β-carbolines from the sample of ayahuasca used in this study are consistent to those already cited in the literature (Callaway, Brito, Neves, 2005; Callaway, 2005; McIlhenny *et al*., 2009; Pires, Oliveira, Yonamine, 2010), except for harmine. It is known that additional tetrahydroharmine may be formed from harmine and harmaline during the ayahuasca preparation (Callaway, 2005; McIlhenny *et al.*, 2009). In fact, tetrahydroharmine was the main alkaloid found in our sample. Although less studied than other β-carbolines, THH is also described as a weaker MAO inhibitor, but it can increase serotonin concentration by inhibiting serotonin reuptake and has longer half-life (Callaway, 2003; Domínguez-Clavé *et al*., 2016).

Ayahuasca when administered as a lyophilizate to mice, either by intraperitoneal route or orally, revealed to be a safe drug since gross behavioral alterations were observed only with dose of 1200 mg/kg (10X the ritualistic), corroborating with previous studies in rodents (Pic-Taylor *et al*., 2015). In fact, ayahuasca is considered safe when used alone in the religious contexts (McKenna, 2004; Gable, 2007). However, the association of any psychotropic drugs must be considered with caution, including plants. Leak (2000) warns that the use of *Hypericum perforatum*, *Piper methysticum* and *Valeriana officinalis* during the perioperative period may affect anesthesia. It is suggested that the anesthesiologists should ask their presurgical patients about any medications they are taking, including herbal supplements and other alternative substances, and should decide about the discontinuation of the supplements when necessary, since these products can interfere with anesthesia and potentially cause complications during surgery (Kaye *et al*., 2007; Whinney, 2009; Abe *et al*., 2014).

The results of this study suggest an interaction between ayahuasca (at doses of 1 to 10 times the used in rituals) with morphine and propofol, verified by the modification of the isolated effects of these drugs. The dose of 12 mg/kg of ayahuasca (0.1X the ritual) was not evaluated in association with morphine or propofol because it presented only minor effects. Ayahuasca at the dose of 1200 mg/kg was able to increase the effect of propofol in the rotarod test and to decrease the propofol-induced sleeping time. The results obtained in the interaction of ayahuasca with propofol seem contradictory, i.e., increased incoordination and decreased sleeping time. However, this result agrees with the personal information of ayahuasca users who report that the decoction produces body relaxation, but induces lack of sleep. Ayahuasca, as a MAOI, increases the central levels

of noradrenaline and serotonin which may increase the activation of the ascending reticular activating system. In our study, ayahuasca did not alter sleeping time and motor coordination *per se*, but altered the propofol effect. Propofol causes fast muscle relaxation but it is not considered a good hypnotic drug, instead, it is employed as a sedative and as an adjuvant in inducing and maintenance of anesthesia (McKeage, Perry, 2003). Since the effects of ayahuasca and propofol interaction were opposite on the two tests, we can hypothesize that these effects may be mediated by different pathways, but our current data is not enough to propose the mechanisms of interaction.

Furthermore, ayahuasca revealed to possess analgesic effect *per se*. Thus, the dose of 1200 mg/kg decreased the number of writhes of mice challenged with acetic acid and decreased the paw licking of mice in the first phase (0-5 min) on formalin test. In this respect it is interesting that extracts of *Peganum harmala*, containing high concentration of harmaline, showed antinociception in the formalin test (Monsef *et al*., 2004), corroborating with our data. The previous administration of 120 or 1200 mg/kg of ayahuasca to mice prolonged the morphine analgesia as measured through the hot plate test. This data would suggest a synergic effect of ayahuasca, perhaps inhibiting the morphine metabolism. Clorgyline, a MAOI, potentiated morphine-induced antinociception in tail flick test, but not in the hot plate test (Kitanaka, Kitanaka, Takemura, 2006). On the other hand, ayahuasca was not able to increase morphine analgesia in formalin test, although it has been active by itself (Figure 5). Because ayahuasca *per se* was not active on thermal nociception and did not alter the intestinal transit, it is possible that ayahuasca acts by a non-opioid mechanism, possibly by anti-inflammatory process. Another hypothesis is that ayahuasca analgesic effect depend on the central serotonergic modulation and/or endogenous opioid release which are reached on specific time points.

The β-carbolines exert a plenty of neurophysiological effects such as competitive inhibition of monoamines uptake, inhibition of Na^+-dependent ATPases, among others (for a review, see McKenna, 2004). Interestingly, harman and harmine attenuated some signs of naloxone-precipitated morphine withdrawal syndrome (Aricioglu-Kartal, Kayir, Uzbay, 2003), what corroborate with the hypothesis that these alkaloids may modulate the opioid system. The authors suggested that the beneficial effects may be mediated by imidazoline receptors, what was later evaluated by Miralles *et al.* (2005) that demonstrated the β-carbolines bind with high affinity to imidazoline I2B receptors. However, further studies are needed in order to evaluate the antinociceptive effect of ayahuasca in the presence and absence of antagonists to better elucidate its mechanism of action. Ayahuasca interactions with propofol in the antinociceptive tests were not evaluated because the analgesic effect after propofol acute administration is too short (Anwar, Abdel-Rahman, 1998; Rahman, Hashim, 2011).

Taken together, the set of results showed that the oral pre-treatment with ayahuasca in doses from 1 to 10 times the used in rituals (120-1200 mg/kg) modified some effects of intraperitoneal administration of morphine and propofol. The use of morphine and propofol by i.p. route in our study limits the translation of our results to humans since the intravenous administration leads to faster and more potent effects. However, some pharmacokinetic interactions cannot be discarded. For instance, the finding that ayahuasca increased and extended the effect of morphine on the hot plate suggests that ayahuasca may reduce its metabolism.

In summary, the present study shows that the lyophilizate of ayahuasca decoction modified the analgesic effects of morphine in mice depending on the test employed. Furthermore, the muscle relaxant effect of propofol in the rotarod was prolonged, while its sedative effect seemed reduced. These data suggest that anesthetists should take some caution when using morphine or propofol in followers of ayahuasca cults undergoing surgical procedures. More studies are needed to better investigate the interactions between ayahuasca and morphine/propofol, and pharmacokinetic studies are suggested to address this question.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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